

2020-05-14

Whole-Genome Sequences of Three Plant Growth-Promoting Rhizobacteria Isolated from *Solanum tuberosum* L. Rhizosphere in Tanzania.

Aloo, Becky

American Society for Microbiology

<https://doi.org/10.1128/MRA.00371-20>

Provided with love from The Nelson Mandela African Institution of Science and Technology



Whole-Genome Sequences of Three Plant Growth-Promoting Rhizobacteria Isolated from *Solanum tuberosum* L. Rhizosphere in Tanzania

Becky N. Aloo,^{a,b} Ernest R. Mbega,^a Billy A. Makumba,^c Ines Friedrich,^d Robert Hertel,^d Rolf Daniel^d

^aNelson Mandela African Institution of Science and Technology, Department of Sustainable Agriculture and Biodiversity Conservation, Arusha, Tanzania

^bUniversity of Eldoret, Department of Biological Sciences, Eldoret, Kenya

^cMoi University, Department of Biological Sciences, Eldoret, Kenya

^dGeorg-August University of Göttingen, Institute of Microbiology and Genetics Department of Genomic and Applied Microbiology & Göttingen Genomics Laboratory, Göttingen, Germany

ABSTRACT We present here the complete genome sequences of plant growth-promoting *Klebsiella* sp. strain MPUS7, *Serratia* sp. strain NGAS9, and *Citrobacter* sp. strain LUTT5, isolated from rhizosphere soils and tubers of potato (*Solanum tuberosum* L.) plants growing in the northern and southern highlands of Tanzania.

Plant rhizospheres have long been investigated and exploited for their plant growth-promoting (PGP) rhizobacteria (1, 2). Potato tubers and rhizosphere soils were sampled from the Tanzanian northern and southern highlands for rhizobacterial isolation (3, 4). *Klebsiella* sp. strain MPUS7, *Serratia* sp. strain NGAS9, and *Citrobacter* sp. strain LUTT5, identified by partial 16S rRNA gene sequencing (5), were selected for whole-genome sequencing.

The strains were grown in Trypticase soy broth (Difco) at $37 \pm 2^\circ\text{C}$ for 24 h in a rotary shaker (130 rpm). Total nucleic acids were extracted with the MasterPure DNA purification kit (Epicentre, Madison, WI, USA) and used for sequence library preparations without further processing. Illumina paired-end shotgun libraries were generated with the Nextera XT DNA sample preparation kit and sequenced using the MiSeq system and reagent kit v.3 (2 × 300 bp) (Illumina, San Diego, CA, USA). For Nanopore sequencing, libraries were prepared using the ligation sequencing kit 1D (SQK-LSK108) and the native barcode expansion kit (EXP-NBD103) (Oxford Nanopore Technologies, Oxford, UK). Sequencing was performed for 72 h on a MinION Mk1B device and a SpotON flow cell R9.4 using MinkNOW software v.19.06.8 (Oxford Nanopore Technologies). The short and long reads were called with the MiSeq control software v.2.6.2.1 and Guppy v.3.2.1., respectively. Read quality assessment and processing were performed with fastp v.0.19.5 (6), resulting in 2,304,340, 2,623,096, and 2,439,948 short Illumina reads and 26,946 (N_{50} , 19.4 kb), 34,866 (N_{50} , 19.9 kb), and 31,389 (N_{50} , 20 kb) long Nanopore reads for *Klebsiella* sp. MPUS7, *Serratia* sp. NGAS9, and *Citrobacter* sp. LUTT5, respectively. All kits were used as recommended by the manufacturers, and default parameters were used for all software unless otherwise specified.

Genome assemblies were performed using the Unicycler v.0.4.8 (7) pipeline with SPAdes v.3.14.0 (8), Racon v.1.4.10 (9), BLAST v.2.2.28+ (10), Bowtie 2 v.2.3.4.3 (11), SAMtools v.1.9 (12), and Pilon v.1.23 (13) and resulted three times in single circular chromosomes. The Unicycler pipeline automatically rotated all genomes, defining *dnaA* as the first protein-coding gene. The average coverage was calculated with Qualimap v.2.2.1 (14); Bowtie 2 v.2.3.4.3 (11) was used for short-read mapping, and Minimap2 v.2.17 (15) was used for long-read mapping. This resulted in 74-, 97-, and 61-fold (Illumina reads) and 121-, 216-, and 208-fold (Nanopore reads) genome mean coverage

Citation Aloo BN, Mbega ER, Makumba BA, Friedrich I, Hertel R, Daniel R. 2020. Whole-genome sequences of three plant growth-promoting rhizobacteria isolated from *Solanum tuberosum* L. rhizosphere in Tanzania. Microbiol Resour Announc 9:e00371-20. <https://doi.org/10.1128/MRA.00371-20>.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Copyright © 2020 Aloo et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Becky N. Aloo, aloobecky@yahoo.com.

Received 9 April 2020

Accepted 22 April 2020

Published 14 May 2020

TABLE 1 Genome features of the strains

Feature ^a	Value for:		
	<i>Klebsiella</i> sp. MPUS7	<i>Serratia</i> sp. NGAS9	<i>Citrobacter</i> sp. LUTT5
Genome size (bp)	5,823,634	5,155,099	5,034,577
GC content (%)	55.16	58.74	52.15
No. of genes	5,447	4,866	4,783
No. of CDS	5,331	4,736	4,661
No. of RNAs	116	130	122
No. of rRNAs	25	22	25
No. of tRNAs	84	88	84
No. of ncRNAs	7	20	13
No. of chromosomes	1	1	1
No. of coding genes	5,277	4,702	4,608
No. of pseudogenes	54	34	53

^aCDS, coding DNA sequences; ncRNAs, noncoding RNAs.

from *Klebsiella* sp. MPUS7, *Serratia* sp. NGAS9, and *Citrobacter* sp. LUTT5, respectively. BLAST analysis of the complete 16S rRNA genes of these strains showed over 99.6% similarity to *Klebsiella grimontii* SB73 (GenBank accession number [NR_159317.1](https://doi.org/10.1093/nar/nkz117)), *Serratia marcescens* NBRC 102204 ([NR_114043.1](https://doi.org/10.1093/nar/nkz117)), and *Citrobacter freundii* ATCC 8090 = MTCC 1658 ([NR_028894.1](https://doi.org/10.1093/nar/nkz117)), respectively. Gene annotation was done with the Prokaryotic Genome Annotation Pipeline v.4.8 (16).

The genome features of the strains are summarized in Table 1. Their protein-encoding genes included potassium, nitrogen, phosphorus, and iron metabolism genes, which are associated with plant growth promotion (17–19). These genomes are the first to be sequenced for potato rhizobacteria in Tanzania and can help to unravel their molecular PGP mechanisms for possible biotechnological application as biofertilizers.

Data availability. The whole-genome shotgun projects of *Klebsiella* sp. MPUS7, *Serratia* sp. NGAS9, and *Citrobacter* sp. LUTT5 have been deposited at GenBank under the accession numbers [CP047604](https://doi.org/10.1093/nar/nkz117), [CP047605](https://doi.org/10.1093/nar/nkz117), and [CP047606](https://doi.org/10.1093/nar/nkz117), respectively. The versions described here are the first versions. The raw sequencing data sets of these strains have been registered in the NCBI Sequence Read Archive database (20) under the accession numbers [SRP255262](https://doi.org/10.1093/nar/nkz117), [SRP255259](https://doi.org/10.1093/nar/nkz117), and [SRP255263](https://doi.org/10.1093/nar/nkz117), respectively.

ACKNOWLEDGMENTS

This work was supported by grants from the German Federal Ministry of Education and Research at the Department of Genomics and Applied Microbiology of the Institute of Microbiology and Genetics at the Georg-August University of Göttingen in Germany. The APC was funded by the L'Oreal-UNESCO Foundation for Women in Science. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

We thank Anja Poehlein of the Georg-August University for sequencing and handling the Illumina data.

REFERENCES

- Kumar A, Singh R, Yadav A, Giri DD, Singh KP, Pandey KD. 2016. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech 6:60. <https://doi.org/10.1007/s13205-016-0393-y>.
- Kumar A, Verma H, Singh VK, Singh PP, Singh SK, Ansari WA, Yadav A, Singh PK, Pandey KD. 2017. Role of *Pseudomonas* sp. in sustainable agriculture and disease management, p 195–215. In Meena VS, Mishra PK, Bisht JK, Pattanayak A (ed), *Agriculturally important microbes for sustainable agriculture*. Springer, Singapore.
- Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato YI, Morisaki H, Mitsui H, Minamisawa K. 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci Plant Nutr* 46:617–629. <https://doi.org/10.1080/00380768.2000.10409127>.
- Aravind R, Kumar A, Eapen SJ, Ramana K. 2009. Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici*. *Lett Appl Microbiol* 48:58–64. <https://doi.org/10.1111/j.1472-765X.2008.02486.x>.
- Frank JA, Reich C, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ. 2008. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol* 74:2461–2470. <https://doi.org/10.1128/AEM.02272-07>.
- Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS,

- Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
9. Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate *de novo* genome assembly from long uncorrected reads. *Genome Res* 27:737–746. <https://doi.org/10.1101/gr.214270.116>.
 10. Altschul S, Gish W, Miller W, Myers E, Lipman D. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
 11. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>.
 12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>.
 13. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <https://doi.org/10.1371/journal.pone.0112963>.
 14. Okonechnikov K, Conesa A, Garcia-Alcalde F. 2016. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics* 32:292–294.
 15. Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* 34:3094–3100. <https://doi.org/10.1093/bioinformatics/bty191>.
 16. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
 17. Ahemad M, Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 26:1–20. <https://doi.org/10.1016/j.jksus.2013.05.001>.
 18. Babalola OO. 2010. Beneficial bacteria of agricultural importance. *Biotechnol Lett* 32:1559–1570. <https://doi.org/10.1007/s10529-010-0347-0>.
 19. Olanrewaju OS, Glick BR, Babalola OO. 2017. Mechanisms of action of plant growth promoting bacteria. *World J Microbiol Biotechnol* 33:197. <https://doi.org/10.1007/s11274-017-2364-9>.
 20. Leinonen R, Sugawara H, Shumway M, International Nucleotide Sequence Database Collaboration. 2011. The Sequence Read Archive. *Nucleic Acids Res* 39:D19–D21. <https://doi.org/10.1093/nar/gkq1019>.